

# “Quantitative High-Throughput Screening (HTS) for Chemical Probe Development and Toxicology Testing”.

**G. Sitta Sittampalam, PhD.**

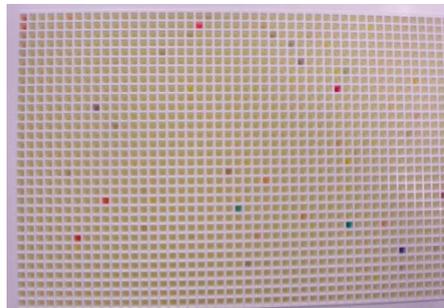
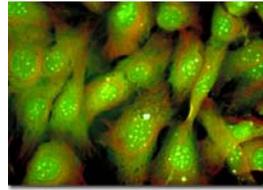
**The NIH Therapies for Rare & Neglected Diseases (TRND) Program  
The NIH Center for Translational Therapeutics (NCTT)**

**NCS Webinar on  
Innovative Technologies for  
Longitudinal Birth Cohort Study  
Assessments  
July 19, 2011**



# What is High-Throughput Screening?

**HTS**  
defined as  
testing of  
>100,000  
chemicals  
per day for  
a biological  
activity



Same cells or gene  
in each well of 1536-  
well plate

+

Different  
chemical in  
each well of  
1536-well plate

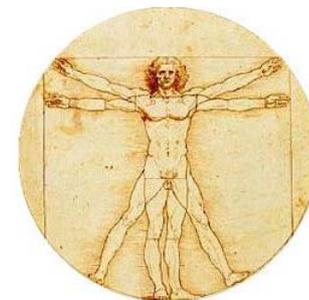
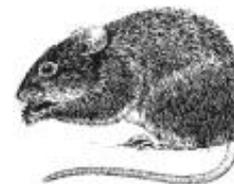
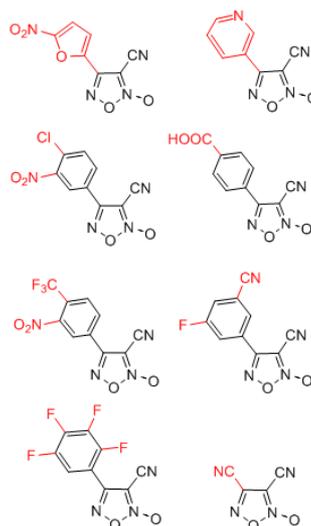
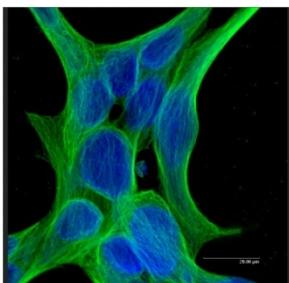
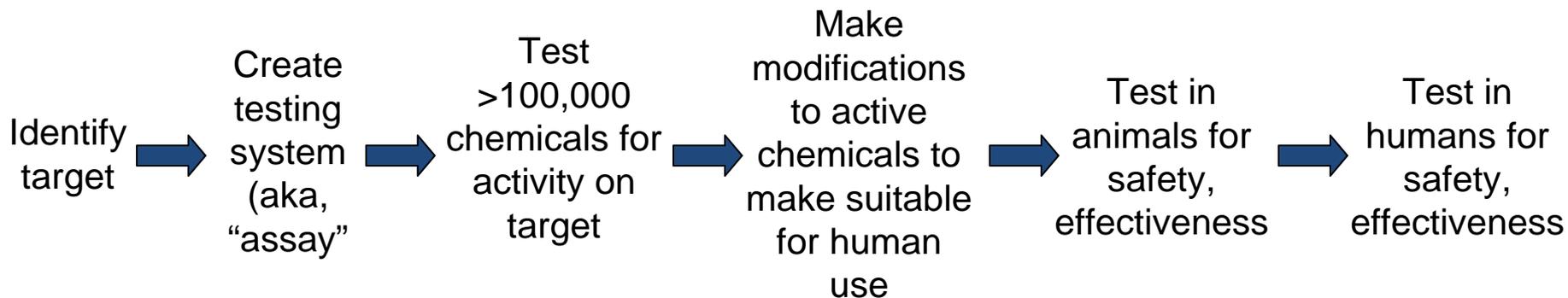
+

Robots, laser  
detectors, and  
computers



Identification of  
which  
chemicals  
affect the cell's  
function

# Steps in HTS & the drug development process



**Same HTS Automation and IT technologies could be adopted for testing large numbers of biological samples**

# HTS assays

*HTS involves a variety of assay types based on:*

a. Purified molecular targets

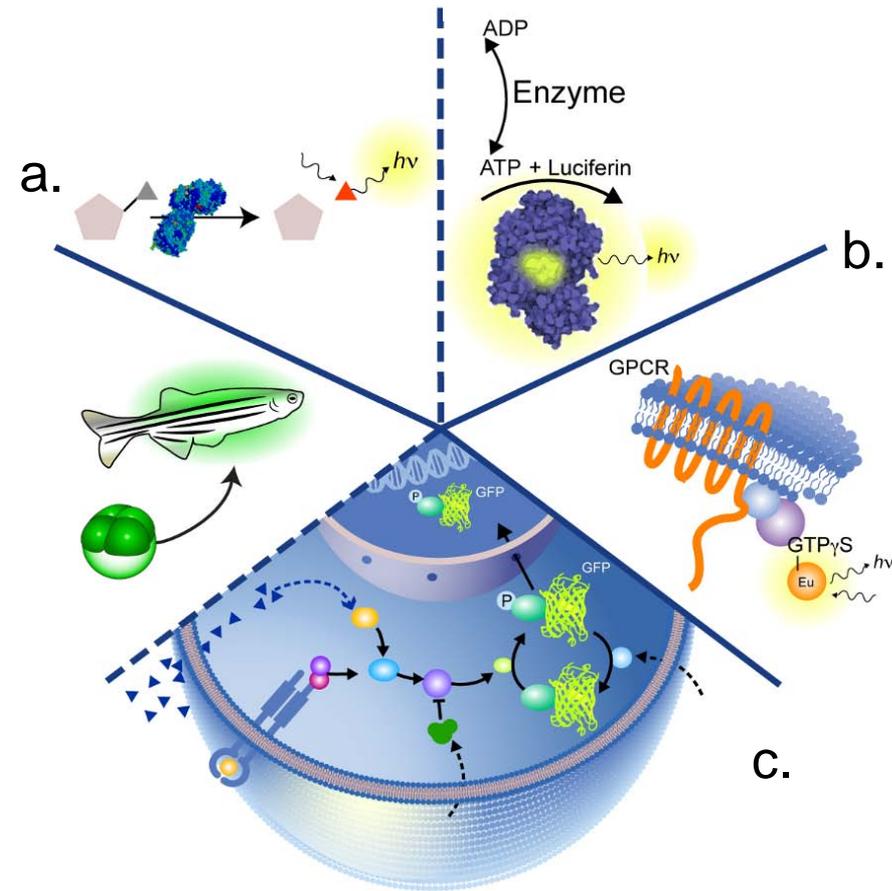
- pro-flourescent substrates
- enzyme cascades
- coupled-enzyme reporters

b. Cell extracts

- membrane preparations

c. Cellular/organism phenotypes

- Reporter-gene
- cellular sensors
- model organisms



- **Assay:** A precisely-defined and efficiently-designed experiment measuring the effect of a substance on a biological process of interest.
- **Robust Assays:** Easily scaled up and automated for HT applications.



>> Assay Guidance

Assay Guidance Manual

Introduction

Assay Validation

Assay Operations for SAR Support

Enzymatic Assays

Receptor Binding Assays

GTPyS Binding Assays

Tissue Culture Assays

Cell-Based Elisa (C-Elisa) and Westerns Blots for Quantitative Antigen Detection

FLIPR™ Assays to Measure GPCR and Ion Channel Targets

Immunoassay Methods

Data Standardization for Results Management

Mechanism of Action Assays for Enzymes

Glossary of Quantitative Biology Terms

NCGC Assay Guidance Criteria

## Table of Contents

Copyright © 2008, Eli Lilly and Company and the National Institutes of Health Chemical Genomics Center. All Rights Reserved. For more information, please review the [Privacy Policy](#) and [Site Usage and Agreement](#).

### How to cite this document:

*Assay Guidance Manual Version 5.0, 2008*, Eli Lilly and Company and NIH Chemical Genomics Center. Available online at: [http://www.ncgc.nih.gov/guidance/manual\\_toc.html](http://www.ncgc.nih.gov/guidance/manual_toc.html) (last accessed [insert date here])

### SECTION I: INTRODUCTION

#### A. INTRODUCTION

### SECTION II: TRANSFER OF VALIDATED ASSAYS

#### A. OVERVIEW

#### B. STABILITY AND PROCESS STUDIES

- Reagent Stability and Storage Requirements
- Reaction Stability Over Projected Assay Time
- DMSO Compatability

#### C. PLATE UNIFORMITY AND SIGNAL VARIABILITY ASSESSMENT

- Overview
- Interleaved-Signal Format
  - Procedure
  - Summary Signal Calculations and Plate Acceptance
  - Spatial Uniformity Assessment
  - Inter-Plate and Inter-Day Tests
  - Summary of Acceptance Criteria
  - 384-well Plate Uniformity Studies
- Uniform-Signal Plate Layouts
  - Procedure
  - Summary Calculations and Plate Acceptance Criteria

# Robust Assay Development Guide

## Development of Robust Experimental Assay Methods (aDREAM) Conference Washington, DC - September 27, 2010 (First Inaugural Conference)

Kellogg Conference Hotel at Gallaudet University  
800 Florida Ave. NE. Washington DC. 20002-3695

Sponsored by NIH Roadmap Molecular Libraries Program  
U13 Grant mechanism  
Satellite Conference with Probe Discovery, Ion Channel Focus  
September 28-29, 2010  
Select BioSciences 2010 Series

- Invitation to attend Editor's meeting of the NCGC Assay Guidance Manual: (<http://assay.nih.gov>).
- Share best practices in quantitative biology and assay methods to facilitate and improve HTS and lead optimization efforts in drug discovery.
- All scientists interested in contributing new chapters or updating existing chapters are encouraged to attend.
- Three (3) Minority travel awards for graduate students and postdoctoral students are available. Please visit <http://assay.nih.gov> for information & application forms.

Electronic Registration: <http://www.selectbiosciences.com/conferences/PD2010>  
Note: Limited capacity for attendance- Please register by September 1, 2010

Contacts: Brenda Richardson: [brichardson@kumc.edu](mailto:brichardson@kumc.edu)  
Sara Spencer: [s.spencer@selectbiosciences.com](mailto:s.spencer@selectbiosciences.com)

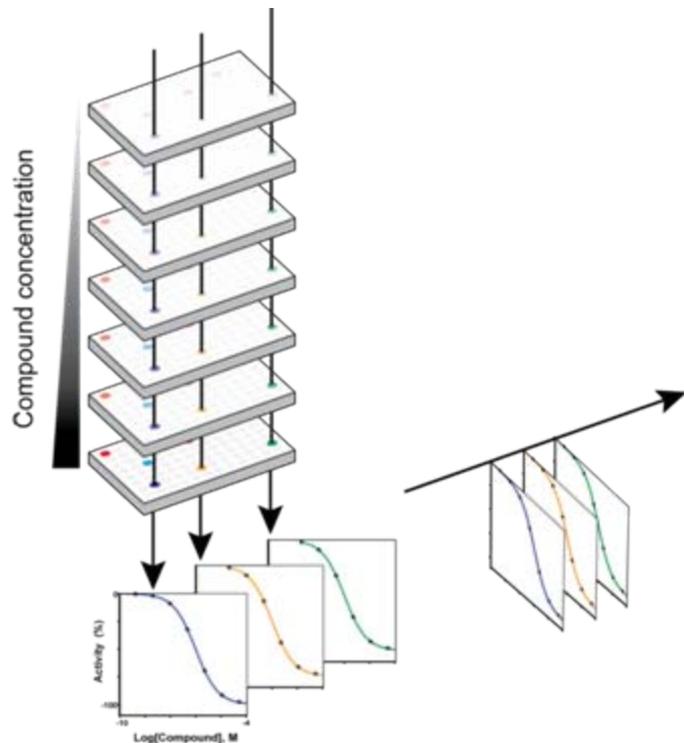
# Assay Guidance Manual

<http://assay.nih.gov/>

# qHTS: Quantitative High Throughput Screening

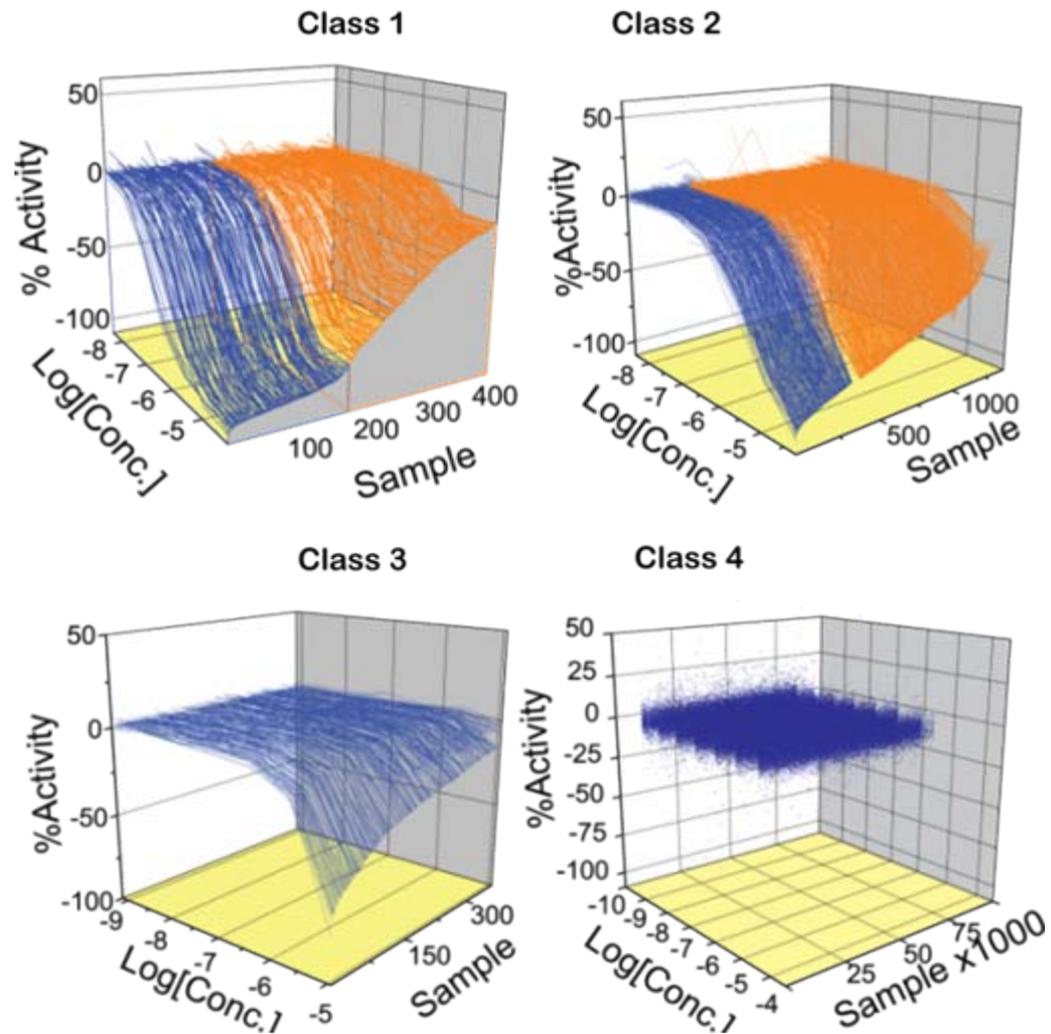
## Concentration Response Screening

- A** Assay concentration ranges over 4 logs (high: ~ 100  $\mu$  M)  
1536-well plates, inter-plate dilution series  
Assay volumes 2 – 5  $\mu$  L



- B** Automated concentration-response data  
Collection ~1 Concentration Response Curve  
(CRC)/sec

- C** Informatics pipeline. Automated curve fitting and classification. CRCs are generated for all 300K samples.



# Take Home Points on HTS Technologies

- High Throughput technologies have matured:
  - Assay technologies,
  - Automation,
  - Data analysis and Informatics.
- These technologies can be applied to any type of sample testing:
  - Small molecules, Peptides, proteins
  - Biological fluids
  - Environmental samples
  - Others..
- Robust and valid assay methodologies are critical.
- These technologies are amenable to miniaturization for field use.
- Require focused effort for reagent/assay development and field expertise.

# The Tox21 Community



NIH CHEMICAL GENOMICS CENTER



# Tox21 Collaboration

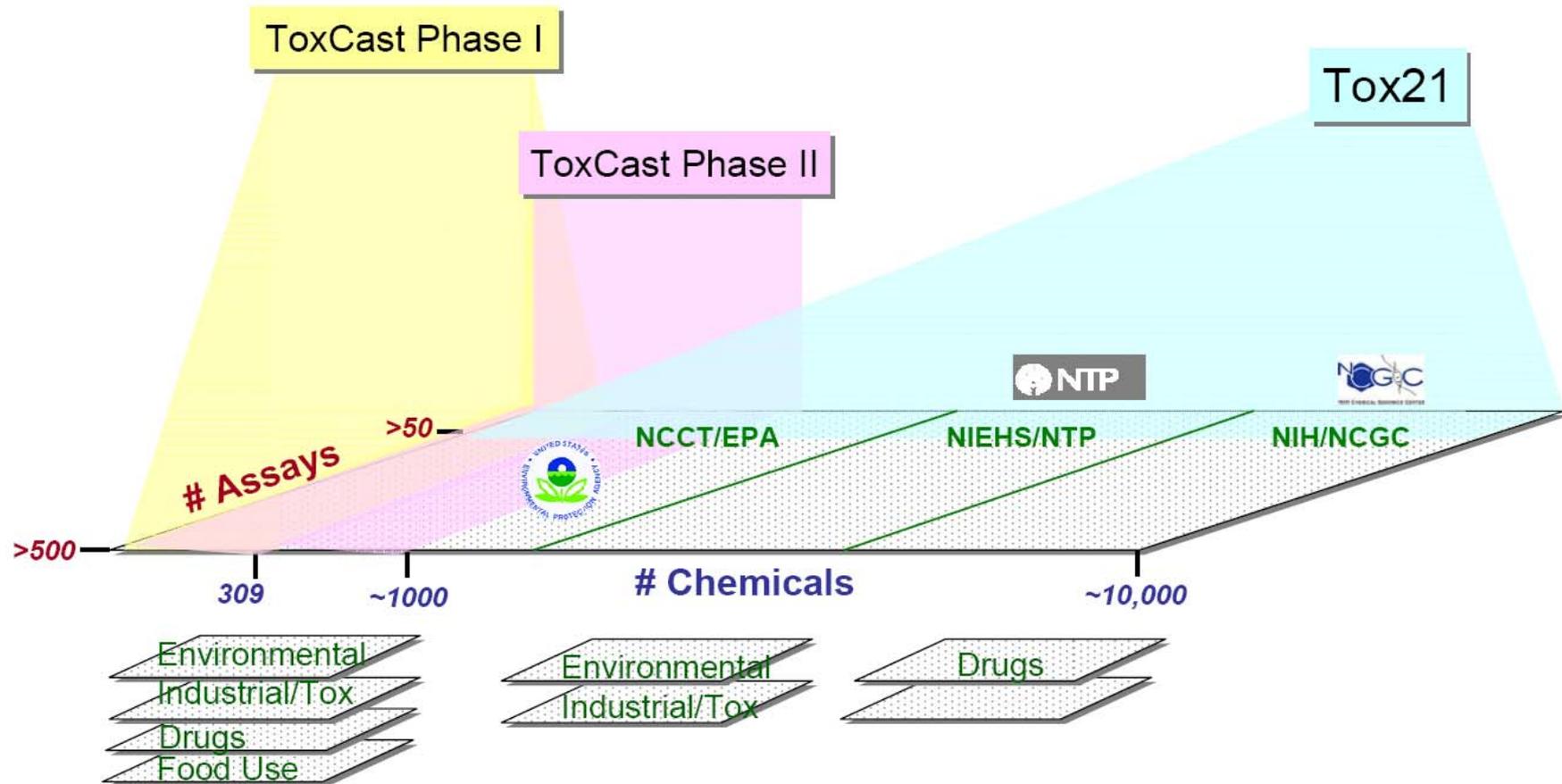
- NTP proposed a Roadmap for the 21<sup>st</sup> century in 2004
  - Development of HTS assays
    - Identification of mechanisms of compound action for further investigation
    - Development of predictive models for biological response
    - Prioritization of substances for further toxicological evaluation
  - Rationale
    - 80,000 chemical compounds registered for use in USA
    - 2000 new compounds introduced into commercial use yearly
    - Rapidly assessment of cellular toxicity of these chemical compounds
- NTP/NIEHS-NCGC collaboration started in 2005
  - NTP 1408 compound collection
  - Validated 1536-well qHTS process using cytotoxicity assays
- EPA joined collaboration in 2006, complementing ToxCast program
  - EPA 1408 compound collection
  - Evaluated a group of nuclear receptor assays
- FDA joined collaboration in 2010
- MOU signed 2008, revised to include FDA 2010
- Long-term goal is to identify *in vitro* chemical signatures that could act as predictive surrogates for *in vivo* toxicity



# The Tox21 Community: Who is doing what?

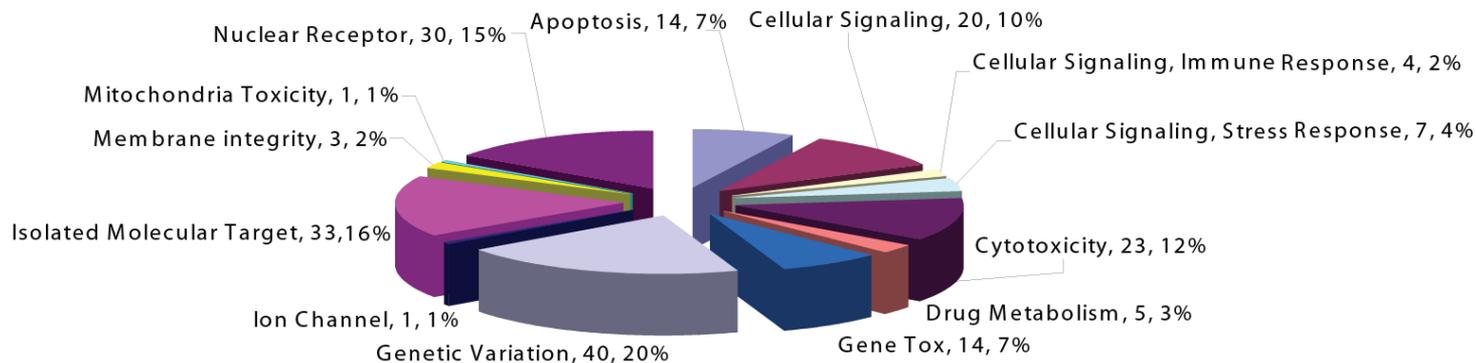
Areas of Expertise	NTP	NCGC	EPA	FDA
Historical Toxicology Data	✓		✓	✓
Human Toxicological Data				✓
Experimental Toxicology	✓		✓	✓
qHTS		✓		
Low to Mid Throughput Assays	✓	✓	✓	✓
Lower Organism Systems	<i>C. elegans</i>		Zebrafish	Zebrafish/ <i>C. elegans</i>
<i>In Vitro</i> 3-D Model Systems	✓		✓	✓
Effect of Human/Animal Genetic Background on Toxic Effects	✓	✓		
Computational Toxicology	✓	✓	✓	✓
Human Exposure Assessment			✓	
Validation Experience	✓	✓	✓	✓ <sup>10</sup>

# Tox21 10K Compound Library



# Assays screened at NCGC against the Tox21Collection

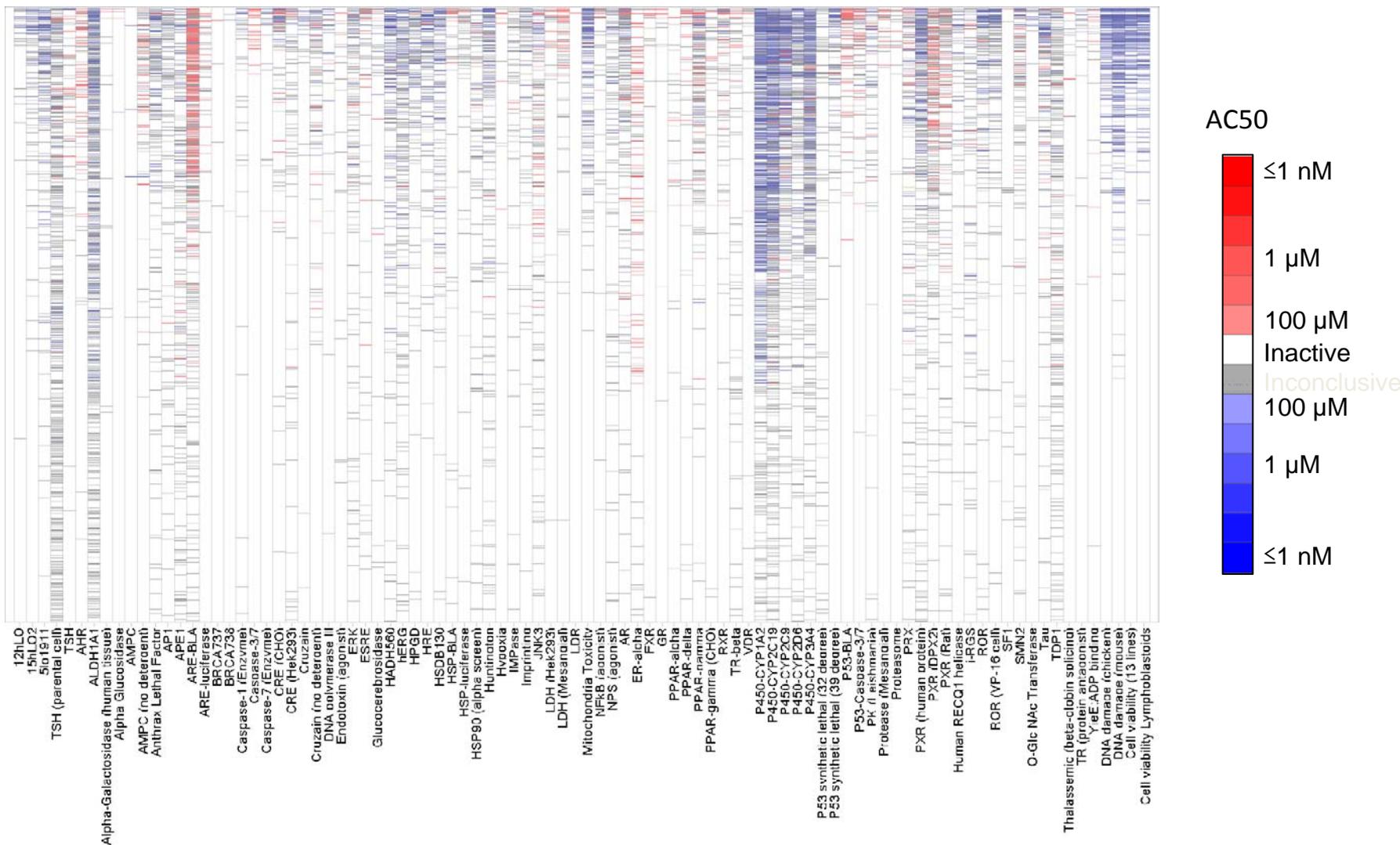
- Phenotypic readouts
  - Cytotoxicity
    - Cell viability assay (measures ATP)
  - Apoptosis
    - Caspase assays (measure activity of Caspase 3/7, 8, 9)
  - Membrane integrity
    - LDH release
    - Protease release
  - Mitochondrial toxicity
    - Mitochondrial membrane potential
  - Gene tox
    - P53, ELG1, DNA damage repair (chicken DT40 lines and mouse lines)
- Cell Signaling
  - Stress response: ARE, ESRE, HSP, Hypoxia, NFkB (agonist), AP-1 (agonist)
  - Immune response: IL-8, TNF $\alpha$ , TTP
  - Other: AP-1, CRE, ERK, HRE, JNK3, NFkB, TSH, LDR, NPS, Proteasome, SF1, SMN2, beta-globin splicing, Anthrax Lethal Factor
- Target specific assays
  - Nuclear receptors: AR, AhR, ER $\alpha$ , FXR, GR, LXR, PPAR $\alpha$ , PPAR $\delta$ , PPAR $\gamma$ , PXR, RXR, TR $\beta$ , VDR, ROR $\alpha$ , ROR $\gamma$
  - hERG channel
  - Isolated molecular targets: 12hLO, 15hLO1, 15hLO2, ALDH1A1, HADH560, HPGD, HSD17b4,  $\alpha$ -Glucosidase,  $\alpha$ -Galactosidase, Glucocerebrosidase, APE1, TDP1, DNA polymerase III, RECQ1 helicase, RGS4, BRCA, IMPase, O-Glc NAc Transferase, Caspase-1 and -7, CBF $\beta$ -RUNX1, PK, Tau, Cruzain,  $\beta$ -Lactamase, PRX, YjeE
- Drug metabolism
  - CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4
- Genetic variation
  - 40 Lymphoblastoid twin cell lines
  - 87 HapMap lines (selected compounds)



# Tox21 Accomplishments

- Phase I (2008-2010): Proof-of-concept studies completed and published using 2800 compound library
  - Compounds profiled (qHTS) in >100 assays
- Phase II (2011- present)
  - 11,000 compound collection assembled including all approved drugs
  - Dedicated robotic platform created that profiles 11K library at 15 concentrations in triplicate in different assay every week
  - Relational map of all pathways operative in human cells created to allow assay prioritization
  - Informatics tools created for data analysis, dissemination, and predictive *in vitro* signature model-building
  - Targeted testing of compounds to follow up primary *in vitro* signatures and test their predictiveness *in vivo*

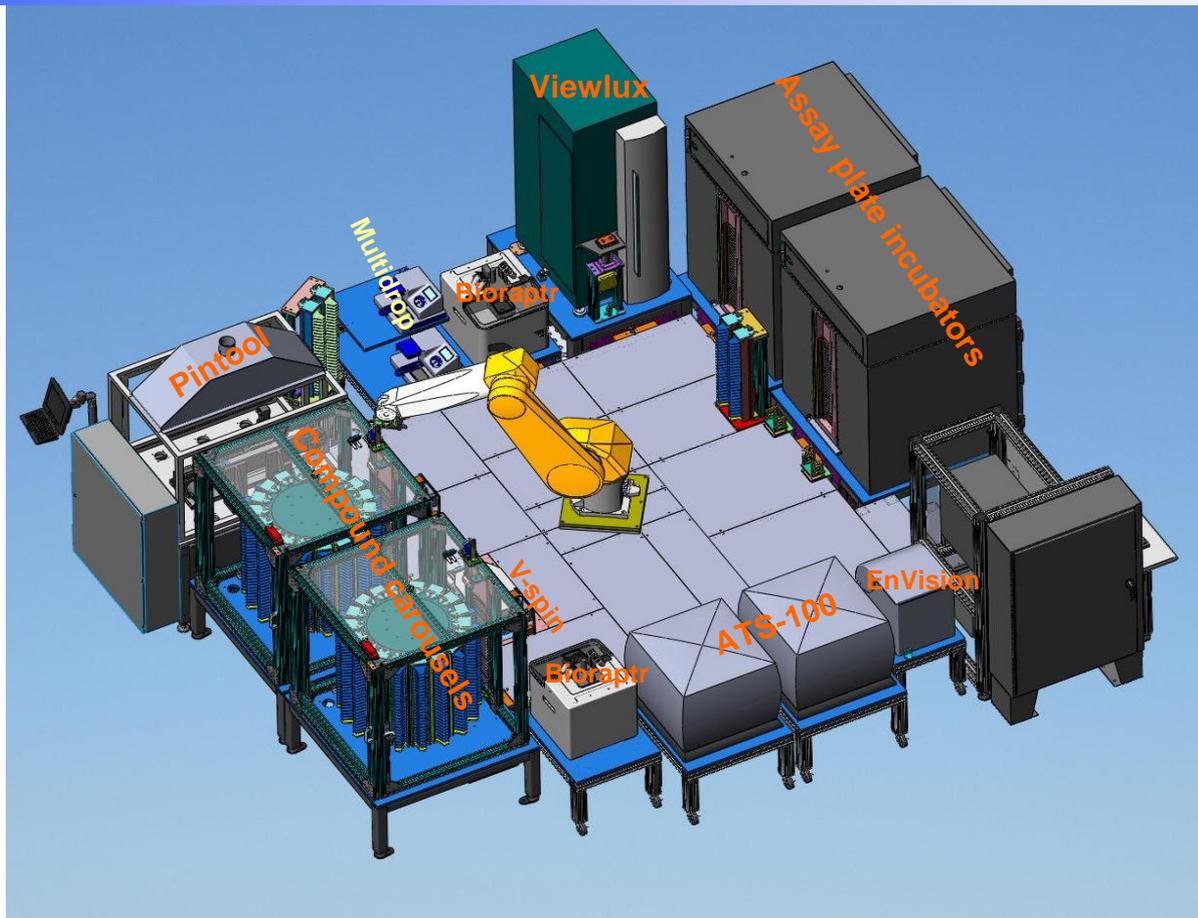
# Tox21 Phase I Compound Activity Profiles



Compounds screened: NTP-1408 and EPA-1462

# Tox21 Robotic Screening System

*To be delivered Feb2011*



**Same HTS Automation and IT technologies could be adopted for testing large numbers of biological samples**

## Talking Points for NCS Longitudinal Cohort Study

- Biological parameters predictive of NCS study goals: toxins, biomarkers, cellular physiology & morphology and necessary clinical panels.
- Assays to be employed to measure/monitor selected parameters.
- Sampling & samples types to be used: body fluids, environmental samples etc.
- HTS technologies required: automation for sample management and assay implementation.
- Robust assay platform: development, validation, implementation: HTS, field use or both.
- Information technology: data analysis, data bases, interpretation & publication.
- Logistics: project management support for collaboration, budget, and other support activities.

# Further Information

austinc@mail.nih.gov



NCGC.nih.gov



TRND.nih.gov

